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Physiological stress responses and meat quality traits of kids subjected to different pre-slaughter stressors

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ABSTRACT

Crossbred Criollo Neuquino castrated male kids, 6 months of age and 24 kg of live weight, were used to investigate the effects of pre-slaughter stressors on physiological characteristics and meat quality attributes. On four separate days, 16 kids were randomly assigned to one of the four pre-slaughter stressor treatments (4 kids per treatment per day): (A) no stress (control); (B) 24 h of food deprivation (fasting); (C) physical stress of forced exercise by an animal handler for 30 min at approximately 3 km/h (exercise); or (D) psychological stress by placing kids in a pen with barking dogs for 5 min (fear). Fasted goats had greater ($P < 0.05$) hematocrit, urea and total protein concentrations than controls. Exercised kids had greater ($P < 0.05$) cortisol concentration than controls and goats exposed to barking dogs had greater ($P < 0.05$) hematocrit and cortisol concentration compared with controls. Even though the stressors imposed on the kids induced changes in blood constituents typically associated with the stress response, the intensity and/or duration of these stressors had little or no effect on meat quality.

1. Introduction

Pre-slaughter handling has been described as one of the most stressful procedures endured by farm animals (Cockram and Corley, 1991; Wermer and Gallo, 2008). Stress produced by pre-slaughter management causes metabolic changes that can affect meat quality (Fernandez and Tornberg, 1991; Kannan et al., 2003; O'Neill et al., 2006; Muchenje et al., 2009). Meat with high ultimate pH (>5.9), commonly referred to as dark, firm and dry (DFD), is characterized by a dark colour, high water holding capacity and reduced shelf life (Ferguson et al., 2001) and depending on the ultimate pH value it has increased toughness (Purchas and Aungsupakorn, 1993).

Stressors produce a perturbation on the animal's homeostasis, consequently, an adaptive response is triggered to restore balance. Knowles and Warriss (2000) proposed some blood parameters to evaluate different stressors and indicated that the change of a variable over time in an individual animal is an indication of the extent of the response to a stressful or injurious situation. As stated by Ferguson and Warner (2008) more research is required regarding the effect of specific individual pre-slaughter stressors and the interactions between them, the biophysical changes in muscle and the consequential effects on meat quality traits.

Most of the available information on the effects of pre-slaughter handling on meat quality has been conducted using beef, pigs, broilers and sheep (Kannan et al., 1997; Brown et al., 1998; Geesink et al., 2001; Daly et al., 2006; Ferguson et al., 2007; Bond and Warner, 2007). However, there is limited information on the effects of pre-slaughter

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stressors on the physiological responses and meat quality of goats (Kannan et al., 2003).

In a study made by Domingo (2005), 69% of the carcasses of 'Chivito de Veranada' (5–7 month-old kids) slaughtered in a commercial abattoir had 24 h pH values greater than 6.0. This category of kids is produced in extensive systems and animals are herded for long distances from the summer rangelands before being loaded in a lorry. Hence, total transportation time may last 24 h or more followed by a variable lairage period imposing not only physical stress but also a long period of fasting. Furthermore, goats are usually herded with dogs imposing additional stress. Therefore, the aim of the present study was to determine the effects of different known short-term pre-slaughter stressors on blood parameters indicative of stress and meat quality traits in crossbred Criollo Neuquino kids.

2. Materials and methods

2.1. Site description

The study was carried out in the Experimental Farm Pilcaniyeu of INTA in the Río Negro province of Argentina (70°35′21″W and 41°01′42″S) at 970 m above sea level. The site is characterized by shrubby-grassland steppe dominated by *Mulinum spinosum*, *Senecio filaginoides*, *Poa ligularis* and *Stipa speciosa* (Bran et al., 2000). Field work was carried out in April when the maximum and minimum daily temperatures were 15–22 °C and 0.5–9.5 °C, respectively.

2.2. Animals and experimental treatments

Castrated, 3/4 Criollo Neuquino – 1/4 Angora kids ($n = 64$), with the mean age and live weight of 189 days and 24 kg, respectively were used. All kids originated from the same flock, were reared under an extensive rangeland production system and were weaned at an average age of 130 days. Kids were of similar phenotypic characteristics, clinically healthy, and had been vaccinated according to the standard health management practice of the experimental farm.

Animal handling and experimental procedures were conducted in accordance with regulation procedures for animal welfare of the National Service of Animal Health (Servicio Nacional de Sanidad Animal, SENASA) of Argentina. A week before the study live weight of animals was recorded. The experiment was carried out in four different days and 16 kids were randomly assigned to each day (blocking effect). Each day, four of the 16 animals were also randomly assigned to one of the four experimental treatments, constituting a total of 16 groups with four animals each. The term "group" is used to define the four animals subjected the same day to the same treatment. Before starting the treatments, all kids were penned in an open paddock and deprived of food for 6 h with free access to water.

(A) Non-stressed control: kids remained in an open paddock with *ad libitum* access to water.
(B) Fasting: kids were deprived of food, but not water, for a total of 24 h before slaughter.
(C) Exercise: kids were constantly moved for 30 min before slaughter in an open and flat paddock by a livestock handler at an estimated rate of 3 km/h.
(D) Fear: kids were penned with two barking dogs for 5 min before slaughter in an open paddock. Kids and dogs were not allowed direct tactile contact to avoid injury.

It should be noted that food (not water) was withheld for 6 h before the control, exercise and fear stressors were applied. Whereas, the 24 h fasting included the 6 h food withdrawal period that all goats received before treatment application.

2.3. Blood sampling

Blood samples were collected 72 h before (basal value) and immediately after stressor treatment (post-treatment), via jugular venipuncture into vacuum tubes containing 0.117 ml of 15% K_3 EDTA (Becton, Dickinson & Co., Bergen, NJ, USA) by trained personnel. Basal value samples were collected at 09:30 h, whereas post-treatment samples were collected at 10:00, 15:00, 16:30 and 17:30 h for kids in the fasting, control, exercise and fear treatment groups, respectively. Blood tubes were then centrifuged at $1006 \times g$ for 20 min and the resulting plasma was placed into safe-lock microtubes and stored at -20 ± 1 °C pending analysis.

2.4. Physiological indicators

Hematocrit (HEM) was measured, in duplicate, using whole blood, whereas cortisol (CORT), urea nitrogen (PUN), total protein (TP) and creatine kinase (CK) were measured in the plasma also in duplicate. Hematocrit (expressed as a percentage) was determined using micro-hematocrit capillary tubes (Tecnon, Ciudad Autónoma de Buenos Aires, BA, Argentina) and a micro-capillary reader (catalogue number 2201, International Equipment Co., Norfolk, MA, USA). Both PUN and TP were colorimetrically determined using commercially available enzymatic kits according to manufacturer's instructions. The UREMIA test kits (code number: 1810058) used to measure PUN (g/L) and the PROTI2 test kits (code number: 1690001) used to measure TP (g/dL) were manufactured by Wiener Laboratories S.A.I.C. (Rosario, SFE, Argentina) and absorbance was measured using the Spectronic 20D+ spectrophotometer (model number: 33183-000, Thermo Fisher Scientific Inc., Middlesex, MA, USA). Plasma CORT (μg/dL) was determined using the Active Cortisol EIA assay kit (DSL-10-2000; Diagnostic Systems Laboratories, Inc., Webster, TX, USA), and absorbance was measured using a Multiskan PLUS spectrophotometer (Type 314, Labsystems, Helsinki, Finland). Finally, CK (UI/L) was quantified using the CK-NAC UV AA test kit (code number 1009309; Wiener Laboratorios S.A.I.C., Rosario, SFE, Argentina) and a Metrolab spectrophotometer (model number: 2300 Plus, Metrolab, Ciudad Autónoma de Buenos Aires, BA, Argentina).

HEM, CORT and PUN were measured in samples collected on all four days ($n = 64$), but TP and CK were only measured in blood samples collected on the last two days of the experiment ($n = 32$).

2.5. Slaughtering and sample collection

At the conclusion of each stressor treatment and immediately after blood sampling, kids were slaughtered at an experimental abattoir, and carcasses were chilled at 4 ± 1 °C for 5 h, followed by storage at 2 ± 1 °C for 24 h.

Temperature and pH were measured 45 min (Ti and pHi, respectively) and 24 h post-slaughter (Tu and pHu, respectively). Then, the entire *Longissimus thoracis et lumborum* muscle (LTL) was removed from the left carcass sides and refrigerated at 2 ± 1 °C for colour and water holding capacity measurements. A portion of the LTL between the 5th and 13th ribs was removed, vacuum-packaged, aged an additional two days at 2 ± 1 °C, and subsequently frozen.

2.6. Meat quality traits

2.6.1. pH and colour

Muscle pH and temperature were measured according to the methodology suggested by Garrido et al. (2005) in the LTL between the 4th and 5th lumbar vertebra using a Testo pH meter (model number 230, Testo, Ciudad Autónoma de Buenos Aires, BA, Argentina) equipped with a glass pH electrode and a temperature probe.

Meat colour was measured according to the methodology suggested by Albertí et al. (2005). The cross-section of the LTL at the region of the 1st lumbar vertebra was allowed to bloom for 30 min at 2 ± 1 °C before instrumental colour (L^*, a^* and b^*) was measured using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Bergen, NJ, USA) using D65 illuminant and an 8-mm aperture. Two scans were collected from the surface of each LTL, avoiding areas of connective tissue or intramuscular fat.

2.6.2. Water holding capacity

Water holding capacity (WHC) was determined in duplicate on LTL samples removed from the area of the 6th rib according to the compression method described by Pla Torres (2005). A 2.5 g sample was placed on a sheet of filter paper (type 585, Schleicher & Schulle, Dassel, Germany), and compressed for 2.5 min. The area of the moisture ring was measured. This procedure assumes that this area is related to the amount (weight) of the meat free juice. Results are expressed as a percentage of released juice.